SafePharm Laboratories

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ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 1194/039

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QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	29 October 2004	Standard Test Method Compliance Audit
	09 August 2005	Test Material Preparation
	09 August 2005	Animal Preparation
	09 August 2005	Dosing
	15 August 2005	Assessment of Response
	15 August 2005	Necropsy
§	15 September 2005	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 06 JAN 2006

For Safepharm Quality Assurance Unit*

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*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.

..... DATE: 05 JAN 2006

A Sanders
Study Director

CONTENTS

ACUTE ORAL TOXICITY IN THE RAT – ACUTE TOXIC CLASS METHOD

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)

- Method B1 *tris* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

Method. A group of three fasted females was treated with the test material at a dose level of 2000 mg/kg bodyweight. This was followed by a further group of three fasted females at the same dose level.

The test material was administered orally as a suspension in arachis oil BP. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. There were no deaths.

Clinical Observations. There were no signs of systemic toxicity.

Bodyweight. All animals showed expected gains in bodyweight over the study period.

Necropsy. No abnormalities were noted at necropsy.

Conclusion. The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated to be greater than 2500 mg/kg bodyweight (GHS Category 5 >2000 - 5000 mg/kg bodyweight).

ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)

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The rat was selected for this study as it is a readily available rodent species, historically used in safety evaluation studies, and is acceptable to appropriate regulatory authorities. The oral route was selected as the most appropriate route of exposure and the results are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 08 August 2005 and 29 August 2005.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

[redacted]

2.2 Preparation of Test Material

For the purpose of the study the test material was freshly prepared, as required, as a suspension at the appropriate concentration in arachis oil BP. Arachis oil BP was used because the test material did not dissolve/suspend in distilled water.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rats were supplied by Charles River (UK) Ltd, Margate, Kent, UK. On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were eight to twelve weeks of age. The bodyweights fell within an interval of ± 20% of the mean initial bodyweight of the first treated group.

The animals were housed in groups of three in suspended solid-floor polypropylene cages furnished with woodflakes. With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food (Certified Rat and Mouse Diet (Code 5LF2) supplied by BCM IPS Limited, London, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminants that would reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 19 to 25°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

Using all available information on the toxicity of the test material, 2000 mg/kg was chosen as the starting dose.

Groups of fasted animals were treated as follows:

Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Rats
			Female
2000	200	10	3
2000	200	10	3

All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe. The volume administered to each animal was calculated according to the fasted bodyweight at the time of dosing. Treatment of animals was sequential. Sufficient time was allowed between each group to confirm the survival of the previously dosed animals.

The animals were observed for deaths or overt signs of toxicity ½, 1, 2 and 4 hours after dosing and subsequently once daily for fourteen days.

Individual bodyweights were recorded prior to dosing and seven and fourteen days after treatment.

At the end of the observation period the animals were killed by cervical dislocation. All animals were subjected to gross pathological examination. This consisted of an external examination and opening of the abdominal and thoracic cavities for examination of major organs. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

3.3 Evaluation of Data

Data evaluations included the relationship, if any, between the exposure of the animal to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, gross lesions, bodyweight changes, mortality and any other toxicological effects.

Using the mortality data obtained, an estimate of the acute oral median lethal dose (LD_{50}) of the test material was made as shown in the schematic diagram in Appendix 1.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Mortality Data

Individual mortality data are given in Table 1.

There were no deaths.

5.2 Clinical Observations

Individual clinical observations are given in Table 2.

There were no signs of systemic toxicity.

5.3 Bodyweight

Individual bodyweights and weekly bodyweight changes are given in Table 3.

All animals showed expected gains in bodyweight over the study period.

5.4 Necropsy

Individual necropsy findings are given in Table 4.

No abnormalities were noted at necropsy.

6. CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was estimated to be greater than 2500 mg/kg bodyweight (GHS Category 5 >2000 - 5000 mg/kg bodyweight).

Table 1 Mortality Data

Dose Level mg/kg	Sex	Number of Animals Treated	Deaths During Day of Dosing (Hours)				Deaths During Period After Dosing (Days)								Deaths
			½	1	2	4	1	2	3	4	5	6	7	8-14	
2000	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3

Table 2 Individual Clinical Observations

Dose Level mg/kg	Animal Number and Sex	Effects Noted After Dosing (Hours)				Effects Noted During Period After Dosing (Days)													
		½	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2000	1-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = No signs of systemic toxicity

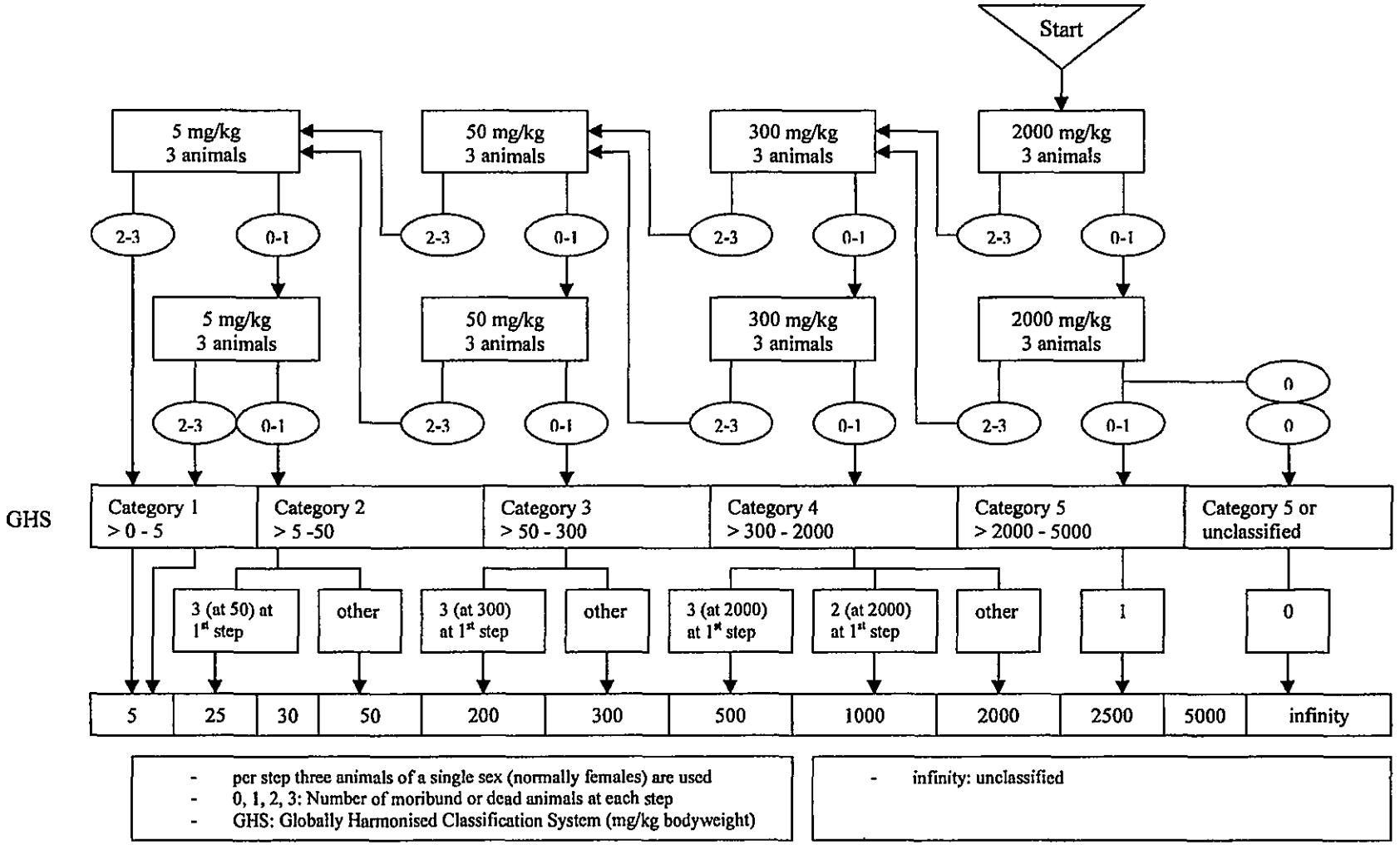
Table 3 Individual Bodyweights and Weekly Bodyweight Changes

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
2000	1-0 Female	229	263	293	34	30
	1-1 Female	232	268	291	36	23
	1-2 Female	200	219	226	19	7
	2-0 Female	220	241	271	21	30
	2-1 Female	237	260	280	23	20
	2-2 Female	223	246	256	23	10

Table 4 Individual Necropsy Findings

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
2000	1-0 Female	Killed Day 14	No abnormalities detected
	1-1 Female	Killed Day 14	No abnormalities detected
	1-2 Female	Killed Day 14	No abnormalities detected
	2-0 Female	Killed Day 14	No abnormalities detected
	2-1 Female	Killed Day 14	No abnormalities detected
	2-2 Female	Killed Day 14	No abnormalities detected

Appendix 1 Test Procedure with a Starting Dose of 2000 mg/kg Bodyweight



Appendix 2 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority